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Project Summary

Effect of Chlorine Dioxide, Chlorite, and Nitrite on Mice With Low and High Levels of Glucose-6-Phosphate Dehydrogenase (G6PD) in Their Erythrocytes

Gary S. Moore and Edward J. Calabrese

Since chlorination of water supplies has come under investigation as a source of trihalomethane formation and possible cancer production, alternative disinfecting substances are being examined. Chlorine dioxide (ClO_2) is a leading possibility for an alternative disinfectant. However, the health consequences of ClO_2 or chlorite (a product formed in the disinfection process) are little known. Further, there is little or no information available on possible interactive effects with other oxidant compounds in the diet or water, such as nitrites. The effect of these compounds might be exaggerated on red blood cells deficient in glucose-6-phosphate dehydrogenase (G6PD). This study reports on these findings.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

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Introduction

The recognition that many public drinking water supplies in the United States contain carcinogenic substances has generated substantial controversy within both the scientific and lay press. The controversy has focused on the occurrence of carcinogenic substances (i.e. trihalomethanes) which tend to be formed in drinking water following the process of chlorination. Epidemiological studies have demonstrated statistical associations between increased cancer mortality and the practice of chlorination of drinking water. These findings have tended to support laboratory studies which demonstrated the occurrence of liver cancer in selected rat and mouse strains exposed to chloroform. For these reasons, the EPA is considering alternative disinfectants to the process of chlorination which would decrease the trihalomethane level in drinking water.

In recognition of the potential widespread utilization of chlorine dioxide as the principal disinfectant in the United States, the intention of this study is to evaluate the health effects of chlorine

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dioxide and a by-product of ClO_2 disinfection, sodium chlorite, on mice with high and relatively low levels of G6PD activity in their erythrocytes.

It was predicted that such an animal would tend to simulate the response of a possible human high risk group to these stressor agents.

Chlorine Dioxide and Sodium Chlorite

There are several methods by which chlorine dioxide may be efficiently produced prior to its application to the drinking water. The two principal ways by which chlorine dioxide is produced includes the following:

a. From chlorine and sodium chlorite
   $Cl_2 + H_2O \rightarrow HOCl + HCl$
   $HOCl + HCl + 2NaClO_2 \rightarrow 2ClO_2 + NaCl + H_2O$
b. From sodium hypochlorite and sodium chlorite
   $NaOCl + HCl \rightarrow NaCl + HOCl$
   $HCl + HOCl + 2NaClO_2 \rightarrow 2ClO_2 + 2NaCl + H_2O$

From a health point of view it is necessary to control the reaction stoichiometry and thereby prevent the release of unintended and unwanted products in the drinking water, e.g. free chlorine, chlorite, and chlorate.

Two recent EPA reports noted that following chlorine dioxide disinfection of surface waters, chlorites made up as much as 50 percent of the chlorine dioxide demand in the pH range of 4.8 to 9.75, while chlorates contributed from 10 to 30 percent of the chlorine dioxide demand within the same pH range. When 1.5 mg chlorine dioxide per liter (mg/1) was added to coagulated, settled, and filtered Ohio River water of 7.1 pH, a chlorite concentration of 0.72 mg/1 and chlorate concentration of 0.41 mg/1 resulted after 42 hours of contact. Therefore, one should expect to find varying quantities of chlorate and chlorite in water which is disinfected with chlorine dioxide. Thus, one must consider the health effects of chlorites and chlorates as well as chlorine dioxide.

Nitrite

Studies on nitrite have demonstrated its ability to produce MetHb, especially in neonates. Research has also indicated that small quantities of nitrite can generate MetHb auto-catalytically without the presence of pre-existing MetHb. This MetHb generated by nitrite can then serve as a catalyst for the formation of more MetHb by chlorite. Chlorite is known to oxidize hemoglobin more readily in the presence of MetHb.

Glucose-6-phosphate Dehydrogenase (G6PD)

In attempting to establish minimal health effect levels of oxidants on erythrocytes, it is important to consider the health effects of such stressors on high risk groups. The two largest groups that fall into this category are persons with lowered G6PD activity and neonates. G6PD deficient cells have a reduced ability to produce NADPH via the pentose phosphate pathway (PPP) and consequently less GSH is formed. Since GSH is the primary mechanism of defense of the red blood cell against oxidant stress, then persons with deficient G6PD levels have a lowered capacity for protection against oxidants. Neonates have a variety of deficiencies and differences in their red blood cells as compared to adults that enhance their susceptibility to oxidant stress and methemoglobin formation. Since nitrates are used in agriculture, and occur naturally in certain food items, there is substantial opportunity for nitrates/nitrites to be ingested and so present additional oxidant stress to erythrocytes. The use of ClO_2 as a disinfectant may exaggerate these effects.

Based upon the hypothesis of the potential health effects that may be caused by chlorite and chlorite plus nitrite combinations on these high risk groups, it is logical to investigate the potential health effects of an oxidant such as ClO_2. The potential use of ClO_2 in public drinking water requires evaluation of ClO_2 alone and in combination with nitrite, with the ultimate significance being the effects of these agents on humans. Due to the overwhelming financial and ethical implications of utilizing human subjects in this study, the most logical possibility lies in the use of an animal model possessing similar enzymatic characteristics as found in the human condition. Therefore, a reasonable assessment might be made concerning the effect of ClO_2 on low level G6PD individuals. The animal models selected for this study were male A/J (high G6PD activity) and C57L/J (low G6PD activity) mice from Jackson Laboratories in Bar Harbor, Maine.

Results

When mice were exposed to chlorine dioxide for 30 days at 100 ppm, there were no significant differences from controls in any of the blood parameters measured. However, significant differences did occur to MCH (mean corpuscular hemoglobin) and MCHC (mean corpusclar hemoglobin concentration) when receiving 50 ppm sodium nitrite in their water for thirty days. There were no strain differences nor were there any additive or synergistic effects between ClO_2 and nitrite.

Both strains of mice exposed to sodium chlorite (100 ppm), or a combination of chlorite and nitrite for 30 days experienced a number of effects on blood parameters. There were no strain versus mouse interactions indicating that the strains did not differ significantly with respect to treatment.

When A/J (high G6PD) and C57L/J (low G6PD) mice were exposed to various levels of sodium chlorite (0.0, 1.0, 10.0, and 100 ppm) for 30 days in their drinking water, there were a number of blood parameters that varied significantly with respect to treatment. However, with the exception of an increase in hemoglobin for the C57L/J strain at the 1.0 and 10.0 level, all significant changes were associated with the 100.0 ppm exposure. There were no significant strain versus treatment interactions for any level of chlorite exposure including 100 ppm.

The results indicate that chlorite produces a number of effects on erythrocytes of both A/J and C57L/J mice. Since chlorite is produced at a level of 50 percent of the chlorine dioxide demand, caution is suggested in setting a standard to insure a sufficient margin of safety. Although there is no significant difference between A/J and C57L/J mice with respect to treatment, the C57L/J (low G6PD) mice may have sufficient G6PD to overcome the oxidant stress of moderate levels of chlorite and/or nitrite. Human G6PD deficients have even less G6PD activity than the C57L/J mice and may be more sensitive than the C57L/J mouse to equivalent amounts of chlorite.

Conclusions

Based upon the data in this report, it appears that exposure of A/J and C57L/J mice to 100 ppm of chlorite for 30 days produces increases in G6PD activity, mean corpuscular volume,

osmotic fragility, acanthocytosis, and cell size (as measured by length [μ] with transmission E.M.). The primary effect of chlorite on erythrocytes appears to be disruption of the cell membrane. There is a slight but significant increase in G6PD activity for both strains with no decrease in GSH. Although GSH is important to protecting the cell against oxidative damage, the presence of GSH within the cell doesn't preclude oxidative damage to the surface membranes. Membrane damage is suggested by the evidence of increased numbers of acanthocytes in the treated mice. Acanthocytes suggest abnormal lipid content of cells and such cells tend to become more permeable with age than normal cells. Membrane damage as an effect of chlorite exposure is further supported by evidence of increased osmotic fragility. Increased osmotic fragility is normally associated with spherocytosis, and the erythrocytes of persons with hereditary spherocytosis (HS) exhibit a characteristic reduction in lipid content with age when compared with normal cells. The HS cell also exhibits an increased rate of Na^+ flux suggesting some leakiness of the membrane. The increased number of acanthocytes along with increased osmotic fragility in this study, therefore, suggest that chlorite has directly or indirectly produced damage to the erythrocyte membrane and probably has caused alteration and/or reduction of certain lipid components of the membrane. The evidence of increased mean corpuscular volume (MCV) and an increased cell length as evidenced by transmission E.M. suggest an influx of fluids to the erythrocyte. It is reasonable to suppose that membrane damage and/or reduction of ATPase activity would encourage an osmotic imbalance and increased fragility of the cell.

Although such changes in erythrocytes would likely reduce the length of survival time for the affected red cells, there was no evidence of a decreased RBC count or an increased number of reticulocytes. Therefore, the effects are not sufficient to produce a hemolytic anemia in the animals tested. Even though it was anticipated the C57L/J strain (low G6PD) would be more susceptible to chlorite ingestion, there were no obvious strain differences.

Thus, it would appear that the mouse model does not reveal the enhanced differential sensitivity at realistic and 10x higher than realistic concentrations to offer an effective means to evaluate the study hypothesis.

The final conclusion is that chlorite exposure in the 100 ppm range produces definite abnormalities of erythrocytes that suggest membrane damage. Such damage may occur in humans with normal G6PD levels when similarly exposed. It is possible that persons with vitamin E deficiency and/or G6PD deficiency may be at increased risk to the effects of chlorite, but this has not been demonstrated in this study. Chlorite may be produced at a rate of 50 percent of the chlorine dioxide demand and levels of 10 ppm have been reported under actual disinfection conditions. Although measurable effects may not be seen at this level, effects may be occurring that are not being measured; or high risk groups may not be part of the population being measured. In any case, the difference of 10 ppm treatment conditions to 100 ppm where effects on erythrocytes are seen represents a safety factor of 10 or less. Normal precaution would suggest a much greater margin of safety when the number of people to be exposed represent a large portion of the population.

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The complete report, entitled "Effect of Chlorine Dioxide, Chlorite, and Nitrite on Mice With Low and High Levels of Glucose-6-Phosphate Dehydrogenase (G6PD) in Their Erythrocytes," (Order No. PB 81-152 381; Cost: $9.50, subject to change) will be available only from:
National Technical Information Service
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